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Mining the $Na_V1.7$ interactome: Opportunities for chronic pain therapeutics

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Abstract

The peripherally expressed voltage-gated sodium $Na_V1.7$ (gene *SCN9A*) channel boosts small stimuli to initiate firing of pain-signaling dorsal root ganglia (DRG) neurons and facilitates neurotransmitter release at the first synapse within the spinal cord. Mutations in *SCN9A* produce distinct human pain syndromes. Widely acknowledged as a “gatekeeper” of pain, $Na_V1.7$ has been the focus of intense investigation but, to date, no $Na_V1.7$-selective drugs have reached the clinic. Elegant crystallographic studies have demonstrated the potential of designing highly potent and selective $Na_V1.7$ compounds but their therapeutic value remains untested. Transcriptional silencing of $Na_V1.7$ by a naturally expressed antisense transcript has been reported in rodents and humans but whether this represents a viable opportunity for designing $Na_V1.7$ therapeutics is currently unknown. The demonstration that loss of $Na_V1.7$ function is associated with upregulation of endogenous opioids and potentiation of mu- and delta-opioid receptor activities, suggests that targeting only $Na_V1.7$ may be insufficient for analgesia. However, the link between opioid-dependent analgesic mechanisms and function of sodium channels and intracellular sodium-dependent signaling remains controversial and disputed. Thus, additional new targets - regulators, modulators - are needed. In this context, we mine the literature for the known interactome of $Na_V1.7$ with a focus on protein interactors that affect the channel’s trafficking or

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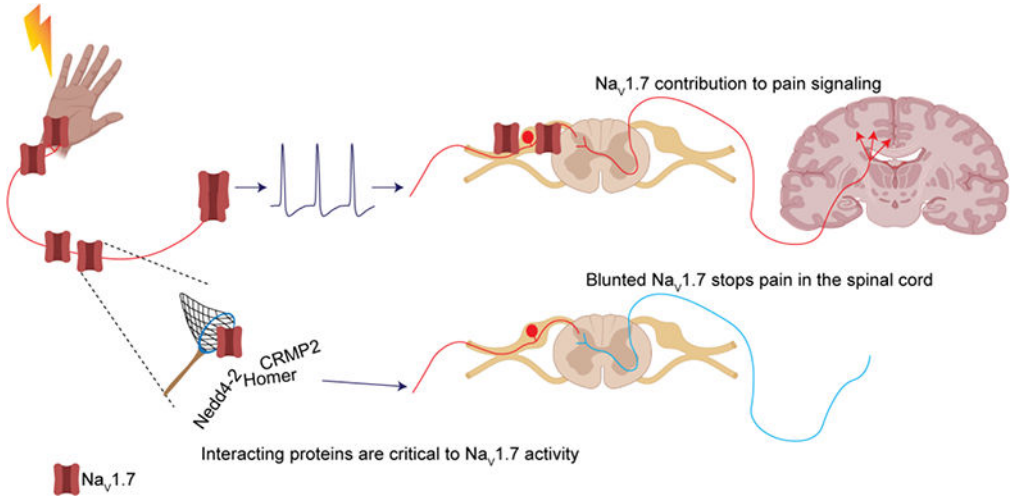
link it to opioid signaling. As a case study, we present antinociceptive evidence of allosteric regulation of $Na_V1.7$ by the cytosolic collapsin response mediator protein 2 (CRMP2). Throughout discussions of these possible new targets, we offer thoughts on the therapeutic implications of modulating $Na_V1.7$ function in chronic pain.

Graphical Abstract



1. $Na_V1.7$ – an introduction to the “gatekeeper” of pain

Physiological pain is largely unpleasant and results from actual or potential tissue damage. The emotional and sensory experience of pain is recognized by the International Association for the Study of Pain as a key response that warns of ensuing danger. Chronic pain, however, contrasts with the biological usefulness of physiological pain, and persists past the point of normal healing to adversely affect 20% of the world’s population [1]. In the United States, chronic pain strains the economy to the value of 635 billion dollars per year [2], exceeding annual costs of several priority health conditions: heart disease ($309 billion), cancer ($243 billion) and diabetes ($188 billion). Inevitably then, pain therapy is an industry requiring considerable attention.

In the last several decades, the voltage-gated sodium channel (VGSC) subtype $Na_V1.7$ has been implicated as an important target in the nociceptive pathway [3, 4]. The protein belongs to a family of VGSCs which gate open in response to voltage and control Na^+ ion influx during the rising phase of the action potentials that underlies all neuronal transmission [5]. Unique gating properties and tissue-level expression patterns and levels of $Na_V1.7$ place the channel in a position to regulate pain signaling [4]. To-date, nine genes coding for voltage-gated sodium channel α pores have been reported – $Na_V1.1$-$Na_V1.9$ [6, 7]. These have been broadly classified by their pharmacology and kinetics with members $Na_V1.1$–$Na_V1.4$ and $Na_V1.6$–$Na_V1.7$ being sensitive to channel block by tetrodotoxin (TTX-sensitive) and displaying rapid inactivation that typically occurs within 5–10 milliseconds. $Na_V1.5$, $Na_V1.8$ and $Na_V1.9$ are TTX-resistant and have much slower inactivation kinetics that produce persistent currents for up to several hundred milliseconds [8].

Dysfunction of some sodium channels, including $Na_V1.7$, is linked to painful human disorders [9]. Peripheral pain stimuli are transmitted along dorsal root ganglia (DRG) neurons making these long bipolar neurons that span from the extremities to the spinal cord an important target for intervention of pain. Variable expression levels for numerous VGSC isoforms and the diverse types of sensory information conveyed, play a strong role in determining the constituents of a DRG's intracellular molecular biome [10]. Furthermore, differential VGSC expression and sensory input are linked to DRG cell body size. Large diameter (> 30 μm cell body) DRGs are predominately myelinated Aα/β fibers that transmit proprioceptive and touch information. This contrasts with smaller diameter (< 30 μm cell body) DRGs that are predominantly Aδ and C-fibers transmitting pain information. While these sizes are relevant for rat DRGS, this relationship is maintained in human DRGs as well [11]. Small and medium DRGs have lower expression of $Na_V1.1$ and $Na_V1.6$ and very high levels of $Na_V1.7$, $Na_V1.8$ and NaV1.9 [10]. Knowledge of this relationship between DRG size and VGSC isoform expression patterns better informs therapeutic development and allows for drug discovery efforts to more intentionally pursue strategies that limit effects on these acknowledged off-target sites.

$Na_V1.7$ has been identified as the dominant contributor to sodium currents among TTX-S subtype channels in small to medium sized DRGs representing nearly 80% of TTX-S current [12]. High $Na_V1.7$ expression in these cells is correlated by high signal of $Na_V1.7$ immunolabeling in small DRG cell bodies, projections to spinal cord, axons, and peripheral terminals in the dermis [13]. In guinea pigs, small cell body C-fibers exhibited augmented $Na_V1.7$ expression compared to medium or large cell body counterparts [14]. Further examination revealed that this augmented $Na_V1.7$ expression was also predictive of DRGs' nociceptive response, further corroborating $Na_V1.7$'s role as a pain-modifying channel [14].

Inevitably then, NaV1.7 mutations are related to a variety of painful phenotypes in addition to painless ones. Gain-of-function mutations underlie painful diseases like inherited erythromelalgia (IEM), paroxysmal extreme pain disorder (PEPD) [15–17], and a $Na_V1.7$-mediated variety of small fiber neuropathy (SFN) [18, 19]. The exact opposite effect on pain has been observed within patients harboring loss-of-function $Na_V1.7$ mutations. These individuals exhibit congenital insensitivity (CIP) to pain and completely lack thermal and mechanical pain thresholds [20]. In addition to pain insensitivity, $Na_V1.7$ loss-of-function mutations produce anosmia, a loss of sense of smell [21], and variable cases of epilepsy [22]. The high expression of $Na_V1.7$ in nociceptive DRGs and phenotypes of human patients with $Na_V1.7$ mutations underscores the importance of this specific voltage-gated sodium channel subtype in pain and has led many researchers to study $Na_V1.7$ in pain models.

2. Progress in drug discovery targeting $Na_V1.7$ – unfulfilled promises

The historical shortcomings of drugs targeting $Na_V1.7$ has been extensively documented in thorough reviews [23, 24], with most concluding that in regards to therapeutic targeting of $Na_V1.7$, effective drug development remains a challenge, particularly in translating basic discoveries into clinical advances. Here, we echo statements made in Foadi's review [25] in highlighting issues with promiscuity of drugs targeting $Na_V1.7$. While several examples of drugs inhibiting NaV1.7 exist, they also engage other mechanisms of action rendering them

susceptible to unwanted side effects. Compounds that affect $Na_V1.4$ or $Na_V1.5$ can produce side effect on motor control and heart rhythm whereas compounds that affect $Na_V1.1$–1.3 can produce CNS side effects on consciousness [24]. Additionally, while several compounds have been characterized due to their antinociceptive promise based on chemical structural analysis, these sodium channel inhibitors have failed to produce similar analgesic effects in clinical studies [26].

Xenon and Convergence have moved into clinical trials with chemotypes based on a neutral spiro-oxindole group and a weakly basic proline respectively [24]. Specifically, Xenon's Xen907 was derived from a series of structural optimizations initially based on a 3-hydroxyoxindole analogue [27]. From here, the furyl moiety was replaced with a thienyl ring and the β-hydroxyketone was altered by shortening of the methylene spacer to afford a 3-aryl-3-hydroxyoxindole. From this base structure, a series of additional changes were introduced including addition of grignard reagent to afford a diol and triethylsilane-mediated dihydroxylation to make a phenol derivative. Aldol condensation and finally intramolecular cyclization resulted in spiroether Xen907. Convergence's CNV1014802 initially based on a weakly basic proline was altered based on a patented process of alpha-carboxamide pyrrolidine derivatives arriving its current structural form.

AstraZenaca has reportedly obtained several patents covering a diverse chemical series including chromane with effects on $Na_V1.7$, but also other Nav1.x channels. Merck's benzazepinone has shown action on $Na_V1.7$, $Na_V1.8$, and NaV1.5. Pfizer and Icagen in collaboration have published several patents based on acidic and zwitterionic series, including several compounds with efficacy on $Na_V1.7$ channels and one molecule currently in clinical trials [24]. Amgen has described a novel series of compounds, triazine derivatives that are $Na_V1.7$ modulators [23]. Specifically of these, positive results have been shown in Phase II clinical trials for Xenon's XEN402 in topically reducing postherpetic neuralgia and orally for primary erythromelalgia [28]. Pfizer had previously advanced a $Na_V1.7$-interacting compound, PF-05089771 into phase II clinical trials for treating pain from diabetic peripheral neuropathy [29]. In its current complete status after analyzing the data of 141 participants, it appears that participants treated with the Pfizer compound had in average lower weekly pain scores in comparison to those treated with pregabalin or placebo. However, other measured outcomes including prevalence of neuropathic pain symptoms such as burning and tingling as well as ratings of overall improvement status remained insignificantly different. Convergence's CNV1014802 has shown modest positive results for treatment of trigeminal neuralgia [30]. However, recent data has shown the Convergence compound, now renamed as raxatrigine to be non-selective NaV inhibitor, exhibiting action not only on $Na_V1.7$, but also $Na_V1.3$ [31]. Despite promising results, isoform selectivity and biophysical characteristics of many of these compounds remain to be disclosed.

Along similar lines, Genentech reported on a series of aryl sulfonamide inhibitors potentially blocking human $Na_V1.7$ by engaging voltage sensor domain four in its activated conformation [32]. Functional studies using patch-clamp electrophysiology demonstrated isoform-selective high affinity binding; however, the pain blocking potential/relevancy or clinical development status of these molecules is unknown. Finally, Payandeh and Hackos [33] suggest three $Na_V1.7$ binding sites that might offer the highest potential for discovery

and optimization of NaV1.7-selective inhibitors. However, the use of these three extracellular druggable sites remains to be adequately exploited. These strategies inevitably begin to cater to the need for better specific therapies by targeting $Na_V1.7$ without modifying other Na_V-family channels and may offer promise for future clinically available $Na_V1.7$-selective drugs.

3. Transcriptional silencing of NaV1.7 – taming the beast from within

In studying transcriptional and post-transcriptional mechanisms that regulate *SCN9A*, Cox and colleagues discovered a unique mechanism for inhibiting $Na_V1.7$ activity [34]: a natural antisense transcript (NAT) for *SCN9A*. Remarkably, overexpression of the NAT, in vitro specifically, decreased the level of mRNA, protein and peak current of $Na_V1.7$. As the NAT is conserved in the NaV1.7 gene from rodents to humans, the authors postulated that the NAT may play an important role in regulating human pain thresholds and may represent a potential candidate gene for individuals with chronic pain. Surprisingly, however, mRNA levels of *NAT* and *SCN9A* were not increased in models of inflammatory pain [34], despite previous demonstration of elevated $Na_V1.7$ protein levels following injections of inflammatory agents [35]. The discordance in these results may be explained by the demonstration that *NAT* expression is non-detectable in DRGs where there is robust *Scn9a* expression and conversely, in the only neuronal subtype without *Scn9a* expression – the large proprioceptor DRGs, there is relatively high expression of the *NAT*; the DRGs were divided into 11 categories based on single-cell RNA sequencing [36]. As summarized by Koenig, the human NAT has a number of single nucleotide polymorphisms (SNP) linked to *SCN9A* related pain disorders, including three mutations linked to primary erythromelalgia (PE) (A863P, V872G, Q875E), two SNPs linked to congenital insensitivity to pain (CIP) (R896Q, W897X), five mutations linked to CIP (R1599X), PE (A1632T) and PEPD (L1612P) as well as an overlapping phenotype (A1632E) ([37] and references therein). These *SCN9A* point mutations previously shown to cause the human monogenic pain disorders CIP, IEM, and PEPD result in a change in the sequence of the NAT, implying that loss of function mutations in the *NAT* may be responsible for inherited painful disorders linked to mutations in $Na_V1.7$. As another NAT for the voltage-dependent potassium channel *Kcna2* has been reported to be upregulated in response to peripheral nerve injury [38], targeting NATs may represent a new opportunity for designing $Na_V1.7$ therapeutics for pain.

Muroi and colleagues demonstrated the efficacy of adeno-associated virus-based delivery of short hairpin RNA against $Na_V1.7$ silencing excitability in sensory neurons of guinea pigs [39], Cai and co-workers also used a lentiviral-based knockdown of $Na_V1.7$ in Lumbar 5 DRGs to attenuate burn-injury induced mechanical allodynia and thermal hyperalgesia in rats [40], and Fink and others reported the efficacy of non-replicating herpes simplex virus vector-mediated microRNA (miRNA) in reducing cold allodynia, thermal hyperalgesia, and mechanical hyperalgesia in rats with painful diabetic neuropathy [41]. The same group also reported a role for GABA acting through the $GABA_B$ receptor ($G_{i/o}$) on decreasing $Na_V1.7$ levels which may account for why spinal release of GABA (due to DRG neurons overexpressing a GAD expressing vector) reduces pain related behaviors in rats with peripheral diabetic neuropathy [42]. But beyond these reports, silencing of $Na_V1.7$ has not been tried in the clinic.

Evidence also suggests that alternative splicing of exons 5 and 11 regulates $Na_V1.7$ expression. Four alternative splice variants are expressed in human DRG neurons, two of which differ in exon 5 by two amino acids in the S3 segment of domain I (exons 5A and 5N). Two others differ in exon 11 by the presence (11L) or absence (11S) of an 11 amino acid sequence in the loop between domains I and II, an important region for protein kinase A (PKA) regulation. Expression of different splice variants can alter $Na_V1.7$ channel kinetics. After injury, an '11S' variant that lacks 11 amino acids undergoes disproportionate upregulation compared to other variants. PKA phosphorylation of the 11S variant produces a hyper-polarizing shift in $Na_V1.7$ activation, which not only promotes DRG hyperexcitability, but forms part of the response mechanism that upregulates $Na_V1.7$ following an injury [43]. Targeting the splicing machinery may be an opportunity to develop therapeutics with the ideal of favoring proportions of the alternative splice variants towards those facilitating suppression of generation of action potentials, thus contributing to prevention of neuropathic pain.

4. $Na_V1.7$ and opioid signaling – the missing link?

Increasing evidence also mandates attention to pain mechanisms mediated by the complete absence or strong inhibition of $Na_V1.7$ activity. Direct analgesic contributions of endogenous opioid signaling in the absence of $Na_V1.7$ represent one such avenue of interest [44–46]. Until now, most therapeutic strategies have held a narrow scope of targets, centering on only voltage-gated sodium channels or opioid receptors. Some evidence suggests that shifting the focus to opioid signaling apart from the receptor may yield more promising answers, although this view remains contested and some researchers continue to assert that targeting $Na_V1.7$ is sufficient in the battle against chronic pain

Examination of endogenous opioids in the DRGs of $Na_V1.7$-null mutant mice revealed upregulation of both Proenkephalin (*Penk*) mRNA and its downstream protein products in primary afferent sensory neurons [44]. Furthermore, these $Na_V1.7$-null mutant mice exhibited significantly enhanced met-enkephalin immunoreactivity (over twofold increase) compared to wildtype littermates [44]. The enhanced expression of endogenous opioids is correlated to intracellular sodium concentrations such that the loss of $Na_V1.7$ activity promotes opioid production and these opioids act can act on inhibitory G-protein coupled receptors and prevent sensory neuron firing [44]. Interestingly, this phenomenon linking voltage-gated sodium channel and endogenous opioid expression appears constrained to TTX-sensitive sodium channels. Parallel investigation of DRGs in Nav1.8-null mutant mice did not reveal changes in *Penk* mRNA [44]. A complementary analysis of *Penk* expression in wildtype DRGs with pharmacologically inhibited TTX-sensitive sodium channel activity revealed similar results and augmented *Penk* expression [44]. By contrast, introducing the ionophore monensin to increase intracellular sodium levels in wildtype mouse DRGs elicited the opposite effect; this led to *Penk* mRNA downregulation while the housekeeping gene *Gapdh* remained unchanged [44]. These findings reinforce a substantial molecular role for *Penk*-associated protein products' promotion of analgesia in the absence of $Na_V1.7$ activity.

Additional studies support an interaction of $Na_V1.7$ activity and endogenous opioid-dependent pain signaling. In conditional knockout mice with $Na_V1.7$-deficient DRGs,

naloxone administration dramatically reversed analgesia and re-established nociception to levels comparable to littermate controls in both the Hargreaves and Randall-Sellitto tests for thermal and noxious mechanical pain, respectively [44]. Notably, both male and female $Na_V1.7$-deficient mutants demonstrated this trait. This provided the first evidence for translation of endogenous opioid-dependent pain signaling to the behavioral level.

Building on these initial results, investigation of nociceptive behaviors in $Na_V1.7$-null mutants with additional global deletions of μ-opioid receptors (MORs) and δ-opioid receptors (DORs) followed. Mutants deficient in both MORs and $Na_V1.7$ regained minimal nociceptive response to acute heat, but naloxone administration nearly restored their nociceptive response to wildtype levels [46]. This demonstrates that MORs alone cannot account for opioid-dependent analgesia in the absence of NaV1.7. Studying mutants deficient in both DORs and $Na_V1.7$ in parallel revealed similar results, indicating that DORs alone cannot account for opioid-dependent analgesia in the absence of $Na_V1.7$ [46]. Applying the potent selective MOR antagonist CTOP to DOR/$Na_V1.7$-null mutants resulted in an equivalent elimination of the analgesic phenotype compared to naloxone administration to this mutant [46]. Mutants deficient in $Na_V1.7$, MOR, and DOR recapitulated this loss of analgesia, yet, administration of the κ-opioid receptor (KOR) antagonist norbinaltorphimine (norBNI), did not further alter the nociceptive response [46]. Altogether, these data suggest that opioid-dependent analgesia in the absence of $Na_V1.7$ function relies on both MOR and DOR, but not KOR, signaling.

Delving into potential molecular links between $Na_V1.7$ and opioid-associated DNA elements, the transcription factor Nuclear Factor of Activated T Cells 5 (Nfat5) was identified, with five consensus binding sites upstream of the *Penk* coding region. Previous work established the regulation of Nfat5 activity by $Na_V1.7$ and intracellular sodium levels [46]. In experiments examining the effects of intracellular sodium changes, monensin administration decreased *Nfat5* mRNA, while TTX administration increased *Nfat5* mRNA—in addition to these treatments' corresponding effects on *Penk* mRNA [46]. These results point to indirect transcriptional control of *Penk* through intracellular sodium-mediated Nfat5 regulation (Figure 2) [46]. TTX-mediated elevation of *Nfat5* mRNA was absent in $Na_V1.7$-deficient mice, implying that $Na_V1.7$ is the locus of action for this mechanism of endogenous opioid production. Furthermore, *Penk* mRNA expression was increased in Nfat5-deficient mice, and even more so in mutants deficient in both NaV1.7 and Nfat5 [46]. Yet, Nfat5-deficient mice developed normal noxious mechanosensation and thermosensation, despite the augmented *Penk* mRNA expression [46]. These results suggest that loss of NaV1.7 has downstream effects on *Nfat5* and *Penk* which are required events to achieve $Na_V1.7$-targeted analgesia. As a result, therapeutic strategies which target the channel may also need to control *Nfat5* and *Penk* in order to phenocopy Na 1.7-mediated reduction of pain in preclinical models.

At the same time, attempts to replicate some of the previously discussed work have led to contradicting results—particularly in the case of those scrutinizing effects of MOR and DOR-associated endogenous opioid peptide enkephalins on pain phenotypes [47]. In a parallel study within a tamoxifen-inducible $Na_V1.7$ conditional knockout mouse, data revealed similar patterns of *Penk* mRNA expression alteration, but without naloxone-

mediated induction of normal pain thresholds [47]. As a result of these reports, opioid-dependent analgesic mechanisms contributing to electrical signaling or intracellular sodium concentrations, in the absence of $Na_V1.7$, remain controversial and disputed.

Even so, it remains clear that $Na_V1.7$ plays a critical role in pain signaling, despite the complexity of molecular partners that help coordinate its downstream activity. Specifically, observation of altered *Penk* mRNA expression, in response to a $Na_V1.7$-mediated intracellular sodium gradient, further ties $Na_V1.7$ and endogenous opioid signaling together. $Na_V1.7$ also contributes to maintenance of a delicate pro- and anti-nociceptive balance. Disruption of this balance, as in the case of $Na_V1.7$ knockout mice that simultaneously exhibit 5-Hydroxytryptamine receptor 4 ($5\text{-}HT_4$) downregulation and inhibited pronociceptive serotonergic signaling, accompanies aforementioned increases in endogenous opioid levels [48]. Given this, the intertwined fates of $Na_V1.7$ and endogenous opioids in the intracellular landscape of nociceptive regulation ought to guide the scope of future therapeutic strategies. While current approaches may fail to harness this link for augmented benefit in the realm of drug discovery, accounting for the greater $Na_V1.7$ interactome will likely yield a more potent analgesic.

5. The interactome of $Na_V1.7$ – the tail wagging the dog

The identification of $Na_V1.7$ as a molecular target in the battle against chronic pain has been a key milestone. Subsequent efforts to produce clinically adequate $Na_V1.7$ inhibitors have become more specific to the channel and more successful over time, but side effects and specificity still remain significant obstacles. Complexity of the $Na_V1.7$ interactome further increases the difficulty of designing a small molecule inhibitor with the ideal antinociceptive profile. The successful development of both safe and efficacious $Na_V1.7$ inhibitors could benefit from targeting the channels endogenous modifiers. By generating a mouse wherein $Na_V1.7$ is tagged to exogenous protein epitopes that are targets of high-affinity antibodies, purification of the channel together with its interacting partners has recently become possible. Importantly, isolated recordings of tagged $Na_V1.7$ channels and expression of the channels in mice produce wildtype current properties and pain expression [49]. Considering the wide breadth of $Na_V1.7$-interacting partners John Wood and has group have identified by this technique [49], here we consider those proteins already implicated in antinociception of particular interest, or ones with the capacity to further augment an inhibitor’s functional scope (Figure 1).

(A) Homer2

Among the $Na_V1.7$ interactome, the post-synaptic density scaffolding protein Homer2 represents one such interacting partner that also mediates NMDAR signaling, especially in the dorsal horn of the spinal cord [50]. Homer is ubiquitously expressed including in both peptidergic and non-peptidergic DRG neurons and in the spinal cord, in particular in the spinal inhibitory neurons transmitting GABA and/or glycine as well as excitatory glutamatergic neurons. Various isoforms of Homer are expressed in different tissues and other Homer isoforms may also facilitate its nociceptive functions. If interaction between Homer proteins and $Na_V1.7$ stabilize excitatory NMDAR signaling, then this excitatory

signaling could be the first step of a feed-forward process that culminates in $Na_V1.7$ gating and an increase in DRG sensitivity. Evidence indicates that both Homer1b/c and Homer2a/b expression coincidentally increase alongside mGluR5 and GluN2A/B levels in a chronic constriction injury (CCI) model of neuropathic pain [51]. Notably, CCI elicited long-lasting increases in Homer1b/c and Homer2a/b expression within the postsynaptic density of dorsal horn spinal cord neurons in addition to supraspinal structures associated with the cognition of pain—especially the prefrontal cortex and thalamus [51]. This, in parallel with CCI-induced nociceptive behaviors, intimates a role for Homer1b/c and Homer2a/b expression in facilitating nociception. Further support can be derived from exacerbation of CCI-induced mechanical and cold hypersensitivity in a model exhibiting virus-mediated overexpression of Homer1c and Homer 2b, although mutants deficient in both Homer1 and Homer2 demonstrated no significant change in nociceptive capacity [51].

(B) Phosphatidylethanolamine-binding protein 1 (PEBP1)

Considering the significance of opioid-dependent signaling mechanisms in analgesia when $Na_V1.7$ expression is absent, phosphatidylethanolamine-binding protein (PEBP1, alternatively named Raf-1 kinase inhibitor protein or RKIP) is of interest as an endogenous morphine-6-glucuronide-binding (M6G) protein [52]. It is most highly expressed in thyroid and parathyroid glands, adrenal glands, liver, kidney, and testis. There is medium expression in cerebral cortex, hippocampus, caudate, cerebellum. It is also expressed in central canal neurons (GABA), inhibitory neurons of the spinal cord (GABA, Glycine), and excitatory neurons of the spinal cord (Glutamate). There is high expression in peptidergic (neurofilament-positive) DRG neurons, but even higher expression in non-peptidergic DRG neurons. Though there is not much differential developmental expression of PEBP1, there seems to be higher expression of the protein in very aged mice. Data from a bovine chromaffin cell model also show PEBP1 is not only highly resistant to proteolytic degradation, but that PEBP1 binding to M6G functionally acts as a protected molecular vehicle for intracellular transport [52]. However, PEBP1 does not appear to play a role in known mechanisms for MOR-mediated regulation of extracellular signal-regulated kinase signaling [53]. While PEBP1 exhibits direct binding to M6G but not morphine, PEBP1 appears to limit overall morphine metabolism and potentiates the opioid's antinociceptive properties [54]. Targeting this regulator of intracellular Raf-1 kinase signaling could therefore be utilized to enhance opioid potency and reduce the dose required to achieve analgesia.

(C) Fatty acid binding protein 7 (FABP7)

As one amongst a family of cytosolic proteins implicated in lipid transport, FABP7 is known to bind N-acylethanolamines (NAEs), such as the endocannabinoid anandamide, which plays an acknowledged role in antinociception through an association with cannabinoid receptors [55]. FABP7 is largely detected in tissues of the cerebral cortex, hippocampus, caudate, and cerebellum. It is not detected in neurons of the spinal cord, and lowly, but equally in non-peptidergic, neurofilament-positive, and peptidergic neurons. While fatty acid amine hydrolases (FAAHs) and FABPs facilitate NAE catabolism, inhibition of FAAHs and FABPs potentiates NAE signaling to produce anti-inflammatory and antinociceptive effects amongst models of inflammatory, visceral, and neuropathic pain through effects on

cannabinoid receptor 1 (CB_1), cannabinoid receptor 2 (CB_2), and PPARα [56, 57]. If interaction between FABP7 and $Na_V1.7$ promotes FABP7 activity, then targeting this interaction could curb pain. It follows that pharmacological inhibition of FABP7 in both formalin and carrageenan models of inflammatory pain reduced nociceptive behaviors, alongside parallel results in an acetic acid model of visceral pain [58, 59]. However, only relief from thermal, but not mechanical, hyperalgesia was attained by this inhibition in models of neuropathic pain [59]. In the same inflammatory models of pain, administration of CB_1 and PPARα antagonists prior to treatment with FABP inhibitors yielded no indication of analgesia; mice continued to exhibit nociceptive behaviors (both thermal and mechanical hyperalgesia), suggesting that FABP-mediated antinociception harnesses CB_1 and PPARα activity [59]. Interestingly enough, naloxone administration failed to counteract these antagonists, implying that opioid receptors do not play a role in FABP inhibitors' antinociceptive mechanism of action [59].

(D) Mitogen-activated protein (MAP) kinases

Serine/threonine-specific MAP kinases typically link membrane receptor activation to cytosolic signaling cascades [60]. MAP kinases are expressed in all tissues, with high expression in tissues of the cerebral cortex, adrenal gland, appendix, skeletal muscle, lung, bronchus, liver, gallbladder, pancreas, stomach, duodenum, colon, rectum, kidney, urinary bladder, testis, prostate, seminal vesicle, vagina, and placenta. They are also expressed in central canal neurons, excitatory, and more prevalently in inhibitory neurons of the spinal cord. In DRGs, MAP kinases are expressed in neurofilament-positive neurons, as well as in peptidergic and non-peptidergic neurons. In nerve ligation animal models of neuropathic pain, inhibition of ERK, p38, and JNK MAP kinases relieved mechanical allodynia, while p38 inhibition specifically reversed thermal hyperalgesia [61]. Coincident upregulation of p38 and ERK1/2 MAP kinases along with $Na_V1.3$, $Na_V1.7$, and $Na_V1.8$ in painful human neuromas provide additional support and a potential mechanism for MAP kinase-mediated nociception [62]. Interestingly, phosphorylation of $Na_V1.8$ by p38 increases peak current density in DRGs and suggests p38 contributions to not only inflammatory, but neuropathic pain [63]. In models of chronic pain where $Na_V1.7$ expression is increased [64], MAP kinase enhancement of allodynia might be $Na_V1.7$-trafficking dependent.

(E) Filamin A

The actin-binding protein filamin A crosslinks actin filaments while participating in cytoskeleton remodeling to rearrange and anchor membrane proteins [65]. Filamin A is expressed in tissues of the bone marrow, smooth muscle, lung, kidney, endometrium, ovary, and placenta. At low levels, it is also expressed in all subtypes of neurons of the spinal cord, and similarly at low levels in all DRG neuronal subtypes. Filamin A is known to play a role in the skeletal and brain development, as it is involved in building the extensive extracellular matrix. This is supported by a reduction in protein levels in the adult brain. In addition to these functions, filamin A is required for MOR-mediated activation of the MAP kinase p38, and deletion studies indicate that filamin A's MOR-binding site resides on its 24^{th} repeat, near its C-terminal [66]. While the N-terminal of filamin A supports its actin-binding functions, absence of this domain did not obstruct other MOR-associated activity [66]. In the complete absence of filamin A, however, MOR-agonist administration led to a marked

upregulation of MOR expression [66, 67]. By contrast, high-affinity binding of naloxone to filamin A appeared to prevent MOR-dependent opioid tolerance and dependence, according to measurements of Gs coupling and cAMP [68]. Future studies to clarify filamin A-mediated regulation of MOR and to maximize its therapeutic utility are undoubtedly necessary and the extent to which $Na_V1.7$ binding contributes to filamin A activity is of particular interest.

(F) Microtubule-associated protein 2 (MAP2)

MAP2 belongs to the microtubule-associated protein family and functions in both promoting and stabilizing microtubule assembly [69]. MAP2 is expressed highly in tissues of the cerebral cortex, hippocampus, caudate, and cerebellum, and low levels in the pancreas and colon. Though it is expressed in all spinal cord neuronal subtypes, it is more largely expressed in the inhibitory GABA-ergic and glycinergic neurons. In DRGs, while expressed in all neuronal subtypes, it is mostly expressed in neurofilament-positive neurons. Developmentally, higher molecular weight isoforms of MAP2 increase in expression with age. These isoforms of MAP2 also play a role in neuronal cell survival during cell stress. Its enrichment in dendrites implies a role in neuronal development through these morphological structures [69]. In a CCI-induced model of neuropathic pain, rat DRGs exhibited marked downregulation in MAP2 expression, especially at the site of injury [70]. Co-localization of calcium-calmodulin protein kinase II alpha (CaMKIIα) and MAP2 alongside coincident modulation of their expression within the trigeminal subnucleus caudalis in an inferior alveolar nerve transection model of neuropathic pain reinforce MAP2's role in the context of pain signaling [71]. Known functions of MAP2 in tubulin binding also provide a link to the collapsin response mediator family of cytosolic proteins, with more recently described roles in the regulation of voltage gated calcium and sodium channel trafficking [72–74]. The aforementioned shifts in expression within models of neuropathic pain ideally position MAP2 for coordinating a nociceptive response within dendrites. Given coincident expression of MAP2, sodium channels, and calcium channels, it is possible that these channels' expression is linked to MAP2 expression in a directly proportional manner. This theory would suggest that therapeutic approaches limiting MAP2 expression or signaling would proportionally reduce sodium or calcium channel-mediated pain signaling, while potentially facilitating analgesia. The downregulation of MAP2 in CCI-induced neuropathic pain, however, suggests the opposite; MAP2 upregulation may, in fact, prove more beneficial in limiting nociception. From the existing body of knowledge on neuropathic pain, it remains unclear which is a more probable role for MAP2. Further investigation is necessary to clarify this relationship.

(G) E3 ubiquitin ligase Nedd4–2

Neural precursor cell expressed developmentally down-regulated protein 4 (Nedd4–2) belongs to the HECT ubiquitin ligases that function in the ubiquitin proteasome system of protein degradation. The encoded protein contains an N-terminal calcium and phospholipid binding C2 domain followed by multiple tryptophan-rich WW domains and, a C-terminal HECT ubiquitin ligase catalytic domain. It plays a critical role in the regulation of a number of membrane receptors, endocytic machinery components and the tumor suppressor PTEN. Nedd4–2 is highly expressed in tissues of the cerebral cortex, thyroid gland, appendix,

bronchus, gallbladder, pancreas, stomach, duodenum, small intestine, colon, rectum, kidney, prostate, seminal vesicle, fallopian tube, cervix, endometrium, and placenta. It is expressed in the spinal cord neurons at low levels, but at very high levels in peptidergic and non-peptidergic neurons. It has diminished expression in neurofilament-positive neurons. With regard to development, it is necessary for development of the heart, and additionally for neuronal development and homeostasis in the brain. Nedd4–2 monoubiquitinates $Na_V1.7$ to promote channel endocytosis, especially in sensory neurons [64]. Evidence clearly indicates that Nedd4–2 modulates not only $Na_V1.7$ channel expression, but current density and neuronal excitability. It follows that virus-mediated overexpression of Nedd4–2 attenuates $Na_V1.7$ expression and current density, contributing to alleviation of mechanical allodynia in model of neuropathic pain [64]. In addition to the endocytic protein Numb and epidermal growth factor receptor pathway substrate 15 (Eps15), Nedd4–2 also appears to be a requisite participant in $Na_V1.7$'s clathrin-mediated internalization [73]. Specificity of Nedd4–2-mediated $Na_V1.7$ internalization, however, depends on the implication of other cytosolic proteins within the internalization complex—especially collapsin response mediator protein (CRMP2) (see below) [73, 75, 76]. Through modulation of $Na_V1.7$ surface expression, Nedd4–2 indirectly attenuates pain signaling in sensory neurons.

(H) Calretinin

A calcium binding protein involved in calcium signaling and buffering, calretinin (CR) has a demonstrated role in modulation of neuronal excitability and induction of long-term potentiation [77, 78]. CR is highly expressed in tissues of the cerebral cortex, hippocampus, caudate, cerebellum, adrenal gland, testis, and soft tissue. It is expressed, at discernable levels, in inhibitory neurons of the spinal cord and there is very low expression in neurofilament-positive DRG neurons. In the superficial dorsal horn of the spinal cord, CR-expressing neurons have been implicated in processing nociceptive, thermal, itch, and light touch sensations [79]. In early development calretinin may be involved in regulating neuronal migration. "Typical" CR neurons comprise ~85% of total CR-expressing population and behave like excitatory interneurons, with delayed firing discharge and large rapid A-type potassium currents. By contrast, "atypical" CR-expressing neurons comprise the remaining 15% and exhibit traits characteristic of inhibitory interneurons, including tonic firing, initial bursting discharge, I_h currents, and islet cell morphology [79]. Some evidence suggests that the TRPV1-mediated nociceptive response (especially in cases of overstimulation) relies on a calretinin-dependent calcium buffering to protect CR-expressing DRGs from triggering apoptotic mechanisms [80]. It remains unclear, however, whether nociceptive responses mediated by TRPV1, and other pain-associated channels or receptors, require CR-expression. In certain pain phenotypes, enhancement of these calretinin-dependent neuroprotective mechanisms may also serve antinociceptive function by limiting localized calcium signaling near NaV1.7 channels immediately following glutamatergic receptor activation.

(I) Neurotrimin

A member of the IgLON immunoglobulin superfamily, neurotrimin is homologous to opioid-binding cell adhesion molecule (OBCAM) [81]. Neurotrimin is most highly expressed in the cerebellum, but also in lower levels in tissues of the cerebral cortex, lung,

hippocampus, caudate, soft tissue, and skin. It is expressed equally in all spinal cord neuronal subtypes. In DRGs, neurotrimin is mostly expressed TRPM8-expressing peptidergic neurons and a subset of glutamatergic neurofilament-positive neurons. Developmentally, neurotrimin expression migrates from initial expression on un-myelinated axons to largely being at excitatory synaptic contact sites, suggesting a role in synaptogenesis. While very little is known about the pain-associated functions of neurotrimin, OBCAM overexpression in astrocytes has been reported to increase cell size and proliferation through a fibroblast growth factor 2 receptor-mediated pathway [82]. Additional studies are necessary to determine the role of both cell adhesion molecules in pain. Immediately relevant to OBCAM interaction with $Na_V1.7$ is whether binding with the channel modifies endogenous opioid signaling.

(J) Neurofascin

With multiple IGcam and fibronectin domains, neurofascin is an L1 family immunoglobulin cell adhesion molecule that localizes to the axon initial segment (AIS) and nodes of Ranvier [83]. Neurofascin is mostly expressed in tissues of the cerebral cortex and cerebellum. Neurofascin is lowly expressed, if at all, in all neuronal spinal cord subtypes. In DRGs, this protein is mostly expressed in neurofilament-positive neurons. Developmentally, embryonic DRGs mostly express neurofascin with one of the two following domains: a mucin-like domain and a fifth fibronectin type III repeat. Just prior to birth neurofascin proteins co-express the domains. Its expression in the peripheral nervous system posits a role for it in modulating adhesive interactions of DRG neurons with Schwann cells. Notably, high densities of voltage-gated sodium channels cluster at both the AIS and nodes of Ranvier, sites enriched with neurofascin. Unsurprisingly, evidence indicates that sodium channel beta (β) 1 and β3 subunits associate with neurofascin through their extracellular immunoglobulin-like domains [84]. In HEK293 cells, β1 and β3 upregulated heterologously expressed $Na_V1.7$ channels [64]. Given the known role of β proteins in channel kinetics, biosynthesis, trafficking, sub-cellular localization, and cell adhesion, there is also potential for neurofascin to facilitate these functions—all of which are critical in modulating the nociceptive response in neurons. Inhibition of neurofascin, alongside β subunits, to indirectly reduce $Na_V1.7$ surface expression may be valuable.

(K) Fibroblast growth factor 13 (FGF13

Intracellular fibroblast growth factors (FGFs) are not yet well understood but are found in the nervous system [85]. FGF13 is expressed in a small subset of inhibitory GABA-ergic and glycinergic spinal cord neurons as well as a subset of excitatory glutamatergic spinal cord neurons. In DRGs, FGF13 is expressed largely in peptidergic and non-peptidergic DRG neurons, with very low levels being found in neurofilament-positive DRG neurons. FGF13 plays a role in neuroblast polarization and migration in the cerebral cortex and hippocampus. Expression of FGF13 is markedly reduced in the adult brain, with transient expression in cortical neurons during brain development. DRGs maintain high levels of FGF13 expression from development through adulthood [85, 86]. Interestingly, loss of FGF13 in mouse DRGs selectively abolishes heat nociception and prevents sustained action potential firing [87]. Furthermore, FGF13 directly interacts with the C-terminus of $Na_V1.7$, increasing $Na_V1.7$ current density without altering its activation/inactivation properties and maintaining its

surface expression during noxious heat stimulation [87]. Disruption of this interaction reduced heat-evoked action potentials and nociceptive behaviors, marking the FGF13/$Na_V1.7$ complex as a robust target for treating thermal hypersensitivity [87].

(L) Multiple PDZ domain protein (MPDZ) and PDZD2

MPDZ and PDZ2 are present at medium to low expression in all tissues. The proteins are discernably expressed in GABA-ergic central canal spinal cord neurons. In DRGs, this protein is expressed in all DRG neuronal subtypes but more so in peptidergic and non-peptidergic neurons and less so in neurofilament-positive DRG neurons. The MPDZ is primarily expressed in synapses and tight junctions [88]. There, it colocalizes with the scaffolding protein PSD95, and both are thought to be implicated in opioid tolerance and opioid-induced hyperalgesia [89]. Most significantly, heterozygous MPDZ+/− mice exhibited reductions in both of these traits. PDZD2 (PDZ-domain 2 protein) shares some structural traits with MPDZ and was found to bind directly to the intracellular loops of $Na_V1.8$ and $Na_V1.7$ [90]. Interestingly, PDZD2-deficient mice do not exhibit significantly altered nociceptive responses [90]. The precise role of PDZD2-binding to $Na_V1.7$ and $Na_V1.8$ requires further exploration. Therapeutic approaches centering on both MPDZ and PDZD2 may alleviate opioid-induced hyperalgesia while potentially impacting sodium channel surface expression. By targeting $Na_V1.7$ expression specifically within postsynaptic densities, threshold current generation by the channel could be severely blunted by only subtle reduction of total $Na_V1.7$ protein surface expression.

(M) β subunits

The beta subunits are arguably the best-understood accessory proteins of Na_V channels and have been extensively described in several reviews [91–93]. They are expressed in excitable cells of the CNS, PNS, heart and skeletal muscle and are also expressed in non-excitable cells, including astrocytes and radial glia, vascular endothelial cells, and cancer cells. Developmental differences also exist in their expression with β1B and β3 being highly expressed prenatally with decreasing abundance after birth, whereas β1 and β2 expression increases during postnatal development and remains high in adulthood; the developmental time course of β4 expression is unknown, but it is expressed postnatally in rat. In 2013, Laedermann and colleagues showed that the $Na_V1.7$ accessory subunits β1 and β3, but not β2 or β4 associate with $Na_V1.7$ in the ER/Golgi and mediate from there the channels' glycosylation state and membrane expression [94]. Cell-cell interactions and subcellular localization can be mediated by the extracellular domain of β subunits, which contain immunoglobulin-like domains that resemble other families of adhesion molecules. The $Na_V1.7$ accessory protein β2 plays an important role in channel forward trafficking shown by β2-null mice that display reduced $Na_V1.7$ protein expression, decreased TTX-S current densities, and decreased thermal and inflammatory pain thresholds [95]. Koenig and colleagues also identified the β3 subunit in their tandem affinity purified proteomics data and verified the binding to NaV1.7 with reciprocal coimmunoprecipitations from DRG and olfactory bulb [49]. Despite the knowledge of the functional modification of $Na_V1.7$ accessory by β subunits [92, 93], these auxiliary subunits have not been targeted for allosteric regulation.

(N) Collapsin response mediator protein 2 (CRMP2)

Classical roles for CRMP2 include promotion of neurite outgrowth, neuronal polarization, progenitor proliferation, radial migration and microtubule network assembly (see review [96]). CRMP2 is expressed mainly in the nervous system as inferred from positive immunoblotting signals from brain and DRG homogenates while no expression was detected in intestine, lung, liver, kidney, heart, muscle or any other tissues from chicken. Our work has demonstrated CRMP2 to be a major hub for the trafficking of voltage-gated calcium and sodium channels in pain signaling [74, 97–99]. We identified a novel trafficking platform for $Na_V1.7$ driven by hierarchical interactions with post-translationally modified versions of the binding partner CRMP2 [73]; a finding confirmed by the Wood group [49]. The novel binding described between CRMP2 and $Na_V1.7$ was enhanced by conjugation of CRMP2 with small ubiquitin-like modifier (SUMO), and further controlled by the phosphorylation status of CRMP2. We reported that CRMP2 SUMOylation is enhanced by prior phosphorylation by Cdk5 at serine-522 and antagonized by the Src family kinase Fyn phosphorylation at tyrosine-32 [73]. As a result of CRMP2 loss of SUMOylation and binding to $Na_V1.7$, the channel displayed decreased membrane localization and current density, and a commensurate reduction in neuronal excitability. Preventing CRMP2 SUMOylation with a SUMO-impaired CRMP2-K374A mutant triggered $Na_V1.7$ internalization in a clathrin-dependent manner involving the E3 ubiquitin ligase Nedd4–2 and endocytosis adaptor proteins Numb and Eps15 (Figure 3A)[72, 73, 75, 100]. Notably, CRMP2 SUMOylation is increased in neuropathic pain and drives nociceptive behaviors (Figure 3B) via increased $Na_V1.7$ synaptic localization in the dorsal horn of the spinal cord or increased $Na_V1.7$ insertion along the central terminal projection [72]. Using rational design, we identified a heptamer peptide containing the CRMP2 SUMOylation consensus site fused to the transduction domain of the HIV-1 tat protein, called t-CSM, to disrupt the CRMP2-Ubc9 interaction (Figure 3C). This t-CSM peptide inhibited CRMP2 SUMOylation, $Na_V1.7$ membrane trafficking, and specifically inhibited $Na_V1.7$ sodium influx in sensory neurons [76]. Intrathecal injection of t-CSM reversed nerve injury-induced thermal and mechanical hypersensitivity with no sedation or motor impairment in rats [76]. Structural modeling has led to the identification of a pocket harboring CRMP2's SUMOylation motif that, when targeted through computational screening of ligands/molecules, is expected to identify small molecules that will biochemically and functionally target CRMP2's SUMOylation to reduce $Na_V1.7$ currents and reverse neuropathic pain [99]. Mapping of hot spots of CRMP2 surface interaction with SUMOylation machinery may form the basis of future drug discovery campaigns. By disrupting the interaction between CRMP2 and E2 SUMO conjugating enzyme Ubc9, $Na_V1.7$ function can be reduced to offer pain relief [101]. CRMP2 may very well turn out to be a prized target as its additional post-translational modifications allow tuning of functions of another important nociceptive ion channel – the N-type voltage-gated calcium ($Ca_V2.2$) channel. For example, phosphorylation of CRMP2 by cyclin-dependent kinase 5 promotes $Ca_V2.2$ surface expression and augments $Ca_V2.2$ current density [102–105]. Thus, disruption of CRMP2 phosphorylation yields an additional therapeutic target. The generally restricted expression of CRMP2 in DRGs and in the dorsal horn of the spinal cord ideally situate CRMP2 for promoting continuity of pain signals. CRMP2 trafficking of multiple ion channels could allow for dual targeting of calcium and sodium signaling to produce antinociception. Whether the other four members of the CRMP

family couple to $Na_V1.7$ is unknown except for a report demonstrating that CRMP1 interacts with $Na_V1.7$ to mediate retrograde axonal signaling of the axon guidance molecule semaphorin 3A [106].

6. Targeting NaV1.7 - the road not yet taken

With the associated $635B cost of chronic pain to the US economy in 2012 [2] and the country's aging baby-boomer population, chronic pain and novel therapeutics are in need of desperate attention by research and medical communities. As we have highlighted here, $Na_V1.7$ has the correct biological function in gating threshold currents and the correct neuronal expression pattern in pain-sensing dorsal root ganglia sensory neurons to be ideally suited in the targeting of peripheral pain signaling [13]. Initially, similarity to the eight other voltage-gated sodium channels prevented efficient targeting without off-target effects on channels involved in cardiac function and cognition. However, there is reason for optimism that $Na_V1.7$-targeted strategies will soon appear on market with no less than eight pharmaceutical companies and research groups reporting successful mitigation of pain using high-affinity and selective $Na_V1.7$-targeted compounds in preclinical, Phase I, and Phase II clinical trials. Seminal studies by the Waxman group have brought $Na_V1.7$ closer to personalized, genomically-guided treatment of patients with pain [3].

A number of research groups, including our own, have taken an alternative approach and attempted to classify modifiers of $Na_V1.7$ channel expression and functional current levels. These strategies benefit from elucidation of new DRG subtypes separated by RNA sequencing and defining the interactome of $Na_V1.7$. The goals of these studies are to selectively target pain-contributing neurons with minimal off-target effects. The spared nerve injury model of neuropathic pain produces a two-fold increase in $Na_V1.7$ within the sciatic nerve [64]. If an accessory or trafficking protein of $Na_V1.7$ can subtly modify how well the channel is anchored to the membrane, whether the channel can be targeted for degradation, or the rate at which the channel is inserted into the membrane, then pain relief can be achieved. Indeed, several groups have published promising results reversing chronic pain conditions in preclinical models of pain that are incompletely treated by conventional therapeutics.

Among these discussed protein partners of $Na_V1.7$, we are most enthusiastic about targets involved in the dysregulation of $Na_V1.7$ in preclinical models of chronic pain. Increased expression of Homer2, increased SUMOylation of CRMP2, and decreased expression of Nedd4–2 have each been observed in pain models and could be critical events preceding augmentation of $Na_V1.7$ surface trafficking and threshold current production [51, 64, 72]. Targeting these proteins to restore their normal functions could then normalize $Na_V1.7$ in painful diseases that are resistant to conventional therapeutics [2]. Each of these three proteins function at the plasma membrane where they contribute to trafficking or retention of functional $Na_V1.7$. With Na_V family proteins undergoing very slow turnover (roughly 13-day turnover in mice), subtle modifications to $Na_V1.7$ channel trafficking has the potential to produce effective and long-lasting treatments [47, 107].

Clearly, $Na_V1.7$ itself and modifiers of the channel have been validated as targets for control of pain signaling. In the last several years, the pain field has also begun to entertain the possibility that endogenous opioid signaling and $Na_V1.7$ function are fundamentally linked at the level of the spinal cord [44]. This may elegantly allow $Na_V1.7$-targeted approaches to have secondary effects on enhancing endogenous opioid production and promote pain-relieving signaling pathways in DRGs. While incompletely explored and controversial, the link between endogenous opioid production and $Na_V1.7$ DRG expression is another avenue by which to explore the ramifications of targeting $Na_V1.7$-mediated signaling in pain. Carefully mining and traversing the diverging protein-protein roads may make all the difference in successfully developing a therapeutic to target $Na_V1.7$ for chronic pain. The challenges ahead are lovely, dark and deep but given our inescapability from pain, it is imperative that academic and industry keep their efforts on drug discovery around $Na_V1.7$ signaling to fulfill a much-needed promise.

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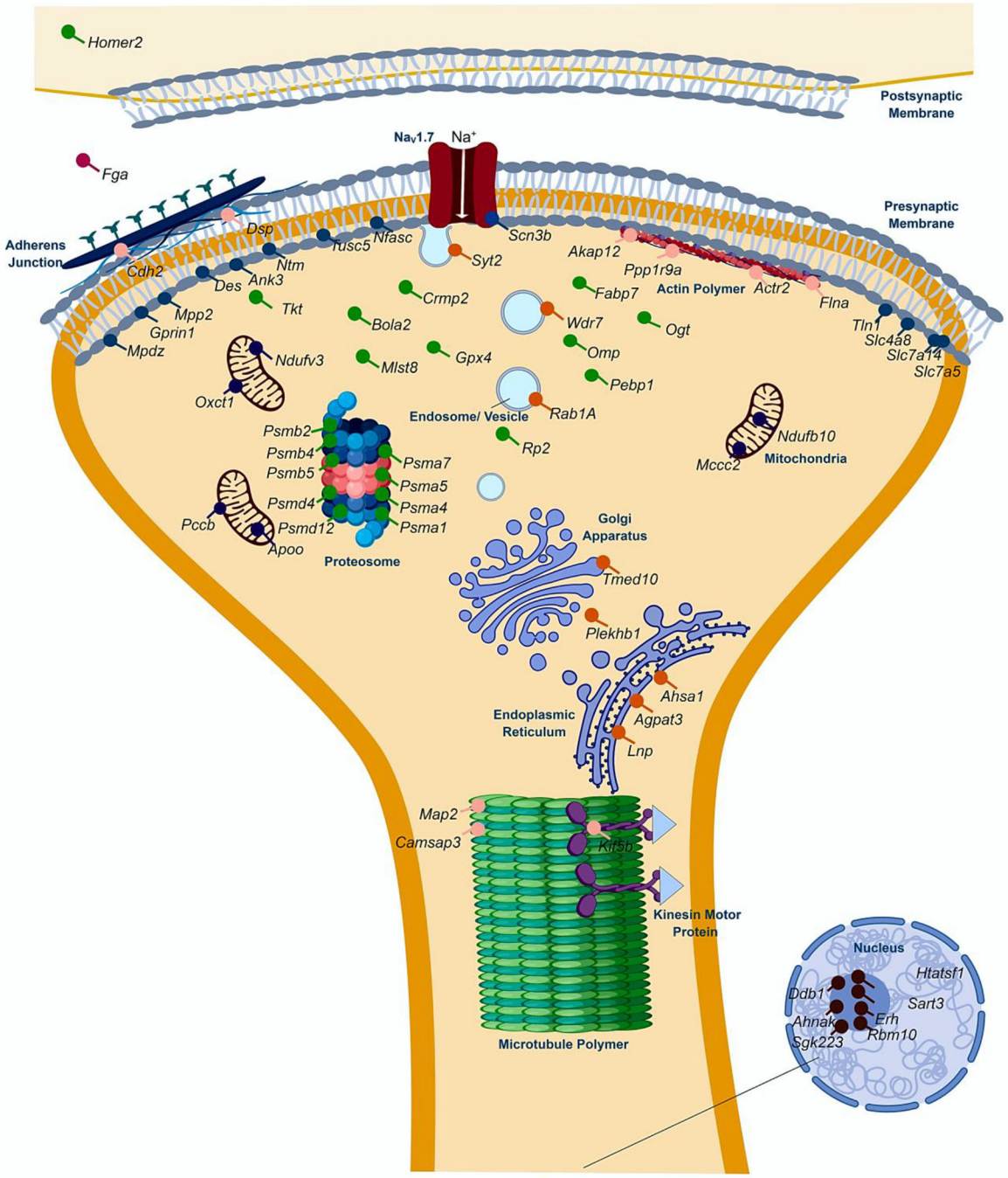

Figure 1. Identified $Na_V1.7$ Protein Interactors.

Schematic of sodium channel NaV1.7 protein interactors including synaptic and intracellular organelle localization. Proteins are identified by gene symbol, in approximate area near a functioning location. Protein abbreviations are as follows and classified by possible functioning locations: *Plasma Membrane (Blue): Scn3b* - Sodium channel subunit beta-3; *Homer2* - Isoform 2 of Homer protein homolog 2; *Rp2* - Isoform 2 of Protein XRP2; *Plekhb1* - Isoform 2 of Pleckstrin homology domain-containing family B member 1; *Tusc5* - Tumour suppressor candidate 5 homolog; *Calb2* - Calretinin; *Tmed10* - Transmembrane

emp24 domain-containing protein 10; *Ntm* – Neurotrimin; *Mpp2* - Isoform 2 of MAGUK p55 subfamily member 2; *Des* - Desmin; *Cdh2* - Cadherin-2; *Ogt* - Isoform 2 of UDP-N-acetylglucosamine; *Nfasc* - Neurofascin; *Mpdz* - Isoform 2 of Multiple PDZ domain protein; *Tln1* - Talin-1; *Dsp* - Desmoplakin; *Slc4a8* - Isoform 2 of Electroneutral sodium bicarbonate exchanger 1; *Slc7a5/Lat1* - Large neutral amino acids transporter small subunit 1; *Ank3* - Ankyrin-3; *Gprin1* - G protein-regulated inducer of neurite outgrowth 1; *Syt2* - Synaptotagmin-2; *Cytoplasm (Green): Bola2* - BolA-like protein 2; *Psma7* - Proteasome subunit alpha type-7; *Psma1* - Proteasome subunit alpha type-1; *Homer2* - Isoform 2 of Homer protein homolog 2; *Psma5* - Proteasome subunit alpha type-5; *Psmb2* - Proteasome subunit beta type-2; *Pebp1* - Phosphatidylethanolamine-binding protein 1; *Plekhb1* - Isoform 2 of Pleckstrin homology domain-containing family B member 1; *Omp* - Olfactory marker protein; *Fabp7* - Fatty acid-binding protein; *Psmb5* - Proteasome subunit beta type-5; *Psmd12* - 26S proteasome non-ATPase regulatory subunit 12; *Mlst8* - Target of rapamycin complex subunit LST8; *Psma4* - Proteasome subunit alpha type-4; *Psmb4* - Proteasome subunit beta type-4; *Psmd4* - Isoform Rpn10B of 26S proteasome non-ATPase regulatory subunit 4; *Rab1A* - Ras-related protein Rab-1A; *Calb2* - Calretinin; *Ahsa1* - Activator of 90-kDa heat-shock protein ATPase homolog 1; *Tkt* - Transketolase; *Ddb1* - DNA damage-binding protein 1; *Des* - Desmin; *Ogt* - Isoform 2 of UDP-N-acetylglucosamine; *Sgk223* - Tyrosine-protein kinase SgK223; *Slc7a5/Lat1* - Large neutral amino acids transporter small subunit 1; *Dpysl2/Crmp2* - Dihydropyrimidinase-related protein 2/ Collapsin Response Mediator Protein 2; *Nucleus (Brown): Bola2* - BolA-like protein 2; *Psma7* - Proteasome subunit alpha type-7; *Psma1* - Proteasome subunit alpha type-1; *Psma5* - Proteasome subunit alpha type-5; *Erh* - Enhancer of rudimentary homolog; *Psmb2* - Proteasome subunit beta type-2; *Psmb5* - Proteasome subunit beta type-5; *Psmd12* - 26S proteasome non-ATPase regulatory subunit 12; *Psma4* - Proteasome subunit alpha type-4; *Psmb4* - Proteasome subunit beta type-4; *Psmd4* - Isoform Rpn10B of 26S proteasome non-ATPase regulatory subunit 4; *Calb2* - Calretinin; *Actr2* - Actin-related protein 2; *Htatsf1* - HIV Tat-specific factor 1 homolog; *Sart3* - Squamous cell carcinoma antigen recognized by T cells 3; *Rbm10* - Isoform 3 of RNA-binding protein 10; *Tkt* - Transketolase; *Ddb1* - DNA damage-binding protein 1; *Des* - Desmin; *Ogt* - Isoform 2 of UDP-N-acetylglucosamine; *Ahnak* - Protein Ahnak; *Sgk223* - Tyrosine-protein kinase SgK223; *Mitochondria (Purple): Ndufv3*; NADH dehydrogenase [ubiquinone] flavoprotein 3; *Ndufb10* - NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10; *Apoo* - Apolipoprotein O; *Oxct1* - Succinyl-CoA:3-ketoacid coenzyme A transferase 1; *Gpx4* - Isoform Cytoplasmic of Phospholipid hydroperoxide glutathione oxidase; *Pccb* - Propionyl-CoA carboxylase beta chain; *Ogt* - Isoform 2 of UDP-N-acetylglucosamine; *Mccc2* - Methylcrotonoyl-CoA carboxylase beta chain; *Cytoskeleton (Light Pink): Flna* - Filamin-A; *Map2* - Microtubule-associated protein; *Actr2* - Actin-related protein 2; *Akap12* - Isoform 2 of A-kinase anchor protein 12; *Mpp2* - Isoform 2 of MAGUK p55 subfamily member 2; *Camsap3* - Isoform 2 of calmodulin-regulated spectrin-associated protein 3; *Ppp1r9a* - Protein Ppp1r9a; *Tln1* - Talin-1; *Dsp* - Desmoplakin; *Ank3* - Ankyrin-3; *Dpysl2/Crmp2* - Dihydropyrimidinase-related protein 2/ Collapsin Response Mediator Protein 2; *ER-Golgi-Endomembrane System -(Orange): Rp2* - Isoform 2 of Protein XRP2; *Apoo* - Apolipoprotein O; *Rab1A* - Ras-related protein Rab-1A; *Ahsa1* - Activator of 90-kDa heat-shock protein ATPase homolog 1; *Wdr7* - WD repeat-containing protein 7; *Tkt* - Transketolase; *Tmed10* - Transmembrane emp24 domain-containing protein 10; *Lnp* -

Protein lunapark; *Agpat3* - 1-acyl-sn-glycerol-3-phosphate acyltransferase gamma; *Extracellular/Synaptic (Dark Pink): Homer2* - Isoform 2 of Homer protein homolog 2; *Fga* - Isoform 2 of Fibrinogen alpha chain; *Apoo* - Apolipoprotein O; *Psmd12* - 26S proteasome non-ATPase regulatory subunit 12

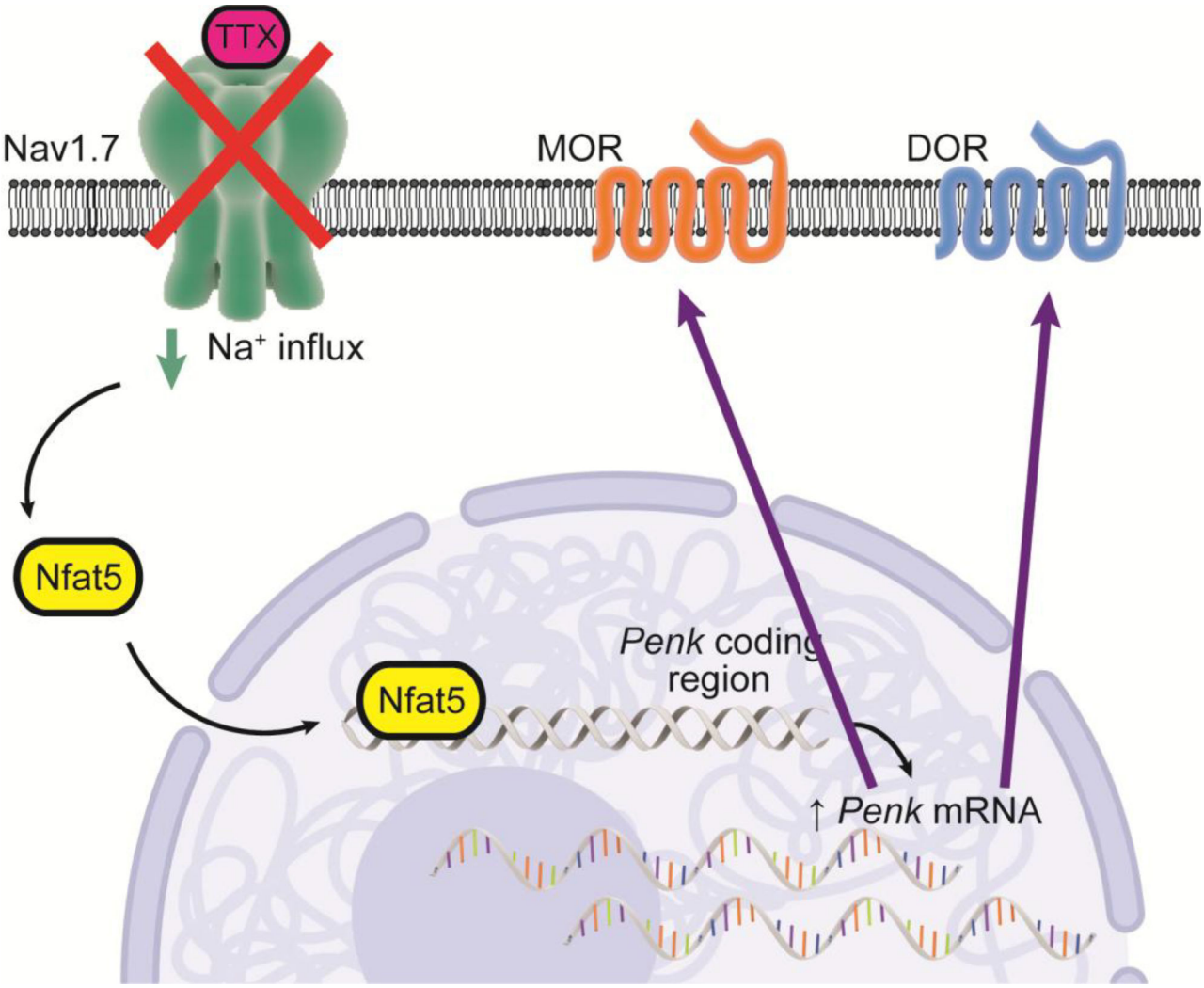

Figure 2. In the absence of $Na_V1.7$ activity, opioid-dependent analgesia relies on Nfat5-mediated facilitation of *Penk* mRNA.

Inhibition of $Na_V1.7$ decreases intracellular sodium levels and leads to upregulation of Nfat5 mRNA and its protein byproduct. Given that Nfat5 binds five consensus binding sites upstream of the *Penk* coding region, Nfat5 likely increases *Penk* mRNA expression. The arrow illustrates that peptides cleaved from *Penk* possibly activating these receptors on neurons (possibly on other cells). Complementary exploration of roles for opioid receptors suggest implication of both MOR and DOR in this analgesic mechanism, but the specifics remain controversial, with reports from distinct groups sometimes bearing contradicting results.

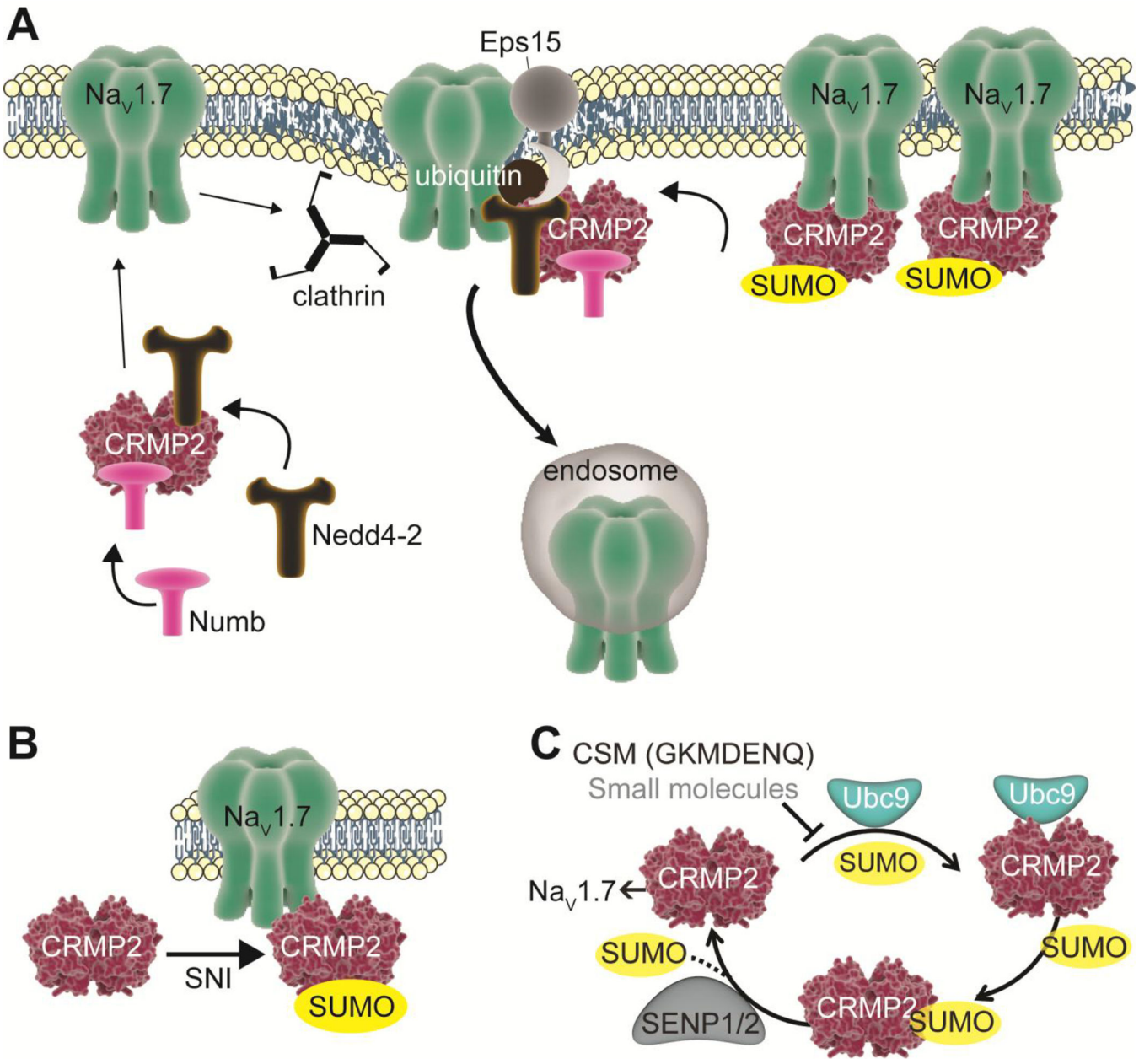

Figure 3. CRMP2 modifications regulate Na$_V$1.7 trafficking in normal and neuropathic pain conditions and prevention of CRMP2 SUMOylation reverses the expression of pain.

(**A**) Numb, a component of the clathrin-mediated endocytosis (CME) machinery, acts as a scaffold between CME and epidermal growth factor receptor substrate 15 (Eps15). Monoubiquitination of NaV1.7 by the E3 ubiquitin ligase Nedd4–2 tags it for endocytosis [64, 73]. Eps15 binds to monoubiquitinylated membrane proteins and induces membrane curvature. CRMP2 SUMOylation is prevented when CRMP2 phosphorylation by Cdk5 at S522 is also prevented [73]. Consequently, non SUMOylated CRMP2 has an enhanced interaction with Numb. The stoichiometry of how many subunits within the CRMP2 tetramer are SUMOylated is unknonwn. (**B**) Cartoon depicting hypothesis of increased CRMP2 SUMOylation in neuropathic pain driving Na$_V$1.7 function. In the unilateral spared

nerve injury (SNI), an injury that involves a lesion of two of the three terminal branches of the sciatic nerve (tibial and common peroneal nerves) leaving the remaining sural nerve intact, increased $Na_V1.7$ protein and CRMP2 SUMOylation was noted in the ipsilateral spinal cord dorsal horn [72]. (**C**) Model of CRMP2 SUMOylation and control of $Na_V1.7$. tat-CSM, cell penetrant version of CRMP2 SUMOylation motif (CSM) decoy peptide. The small ubiquitin-like modifier (SUMO), in a process called SUMOylation, covalently and reversibly tags an ~11 kD SUMO protein to lysine K374 in CRMP2. As with ubiquitination, a cascade of three enzymes, E1 (activating), E2 (conjugating) and E3 (ligase) produce an isopeptide bond between the C-terminal glycine of SUMOs 1–3 and an ε-amino group of a target lysine within a SUMO-binding motif on the acceptor protein. Ubc9 is an E2 enzyme that directly conjugates one or more of the 3 vertebrate SUMO proteins to target proteins, while the sentrin/SUMO-specific proteases (SENP 1 and SENP2) remove SUMOs, thus reversing the modification. A CRMP2 SUMOylation motif (CSM) “decoy” peptide interferes with cellular CRMP2 SUMOylation and decreases $Na_V1.7$ trafficking and currents [76] while intrathecal injection of a cell penetrant version of the CSM peptide reversed nerve injury-induced thermal and mechanical hypersensitivity with no sedation or motor impairment in rats [76]; it is anticipated that small molecules, when developed, will mimic the peptide to achieve a similar silencing of $Na_V1.7$ activity and pain.